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Note

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Estrogen Receptor Gene Analysis in Estrogen Receptor-Positive and Receptor-Negative Primary Breast Cancer

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Background: **In breast cancer patients, about two thirds of the tumors are estrogen receptor (ER)-positive and one third are ER-negative. The molecular mechanisms leading to the ER-negative phenotype are poorly understood. Nearly all ER-negative and about 40% of ER-positive cancers are resistant to endocrine therapy.** ***Purpose:*** **In this study, we examined the entire coding region of the ER gene in ER-positive and ER-negative primary breast tumors to determine whether deletions/insertions or point mutations might account for the ER-negative phenotype.** ***Methods:*** **We amplified exons 1 through 8 of the ER gene in 118 ER-positive and 70 ER-negative primary breast tumors and searched for mutations by single-strand conformation polymorphism analysis, denaturing gradient gel electrophoresis, and DNA sequencing.** ***Results:*** **Both ER-negative and ER-positive tumors contained neutral polymorphisms in codons 10 [TCT→TCC (Ser)], 87 [GCG→GCC (Ala)], 243 [CGC→CGT (Arg)], 325 [CCC→CCG (Pro)], and 594 [ACA→ACG (Thr)]. There was no correlation of any of the polymorphic alleles with the ER phenotype or other clinicopathologic parameters including tumor type, size, grade, or stage. However, the polymorphism in codon 325 showed a strong association with a family history of breast cancer (P = .0005). This association was observed both in premenopausal and postmenopausal patients. Despite extensive searching in exons 1 through 8, we found no deletions/insertions and only two missense mutations in codons 69 [AAC (Asn)→AAG (Lys)] and 396 [ATG (Met)→GTG (Val)] of the same ER-negative tumor. Thus, only 1% of the primary breast cancers had point mutations in the ER gene.** ***Conclusions:*** **In the majority of primary breast cancers, the ER-negative phenotype is not the result of mutations in the coding region of the ER gene, but is due to deficient ER expression at the transcriptional or post-transcriptional level.** ***Implications:*** **The correlation reported previously, as well as our current findings, suggest that further investigations are warranted to understand the possible linkage of the ER gene locus to hereditary breast cancer. [J Natl Cancer Inst 87:446-451, 1995]**

The biological role of estrogens is mediated through high-affinity binding to the estrogen receptor (ER) that belongs to a family of ligand-inducible nuclear receptors that have steroid and thyroid hormones and vitamins as known ligands (*1-3*). The human ER gene is located on chromosome 6q24-q27 (*4*); it extends over more than 140 kilobases, contains eight exons, and is expressed from two promoters, giving rise to two transcripts that differ only in the most 5′ end (*5,6*). Alternative splicing leads to exon 1 (684 nucleotides) or exon 1′ (638 nucleotides), which differ in their most upstream portions, but share the downstream sequence of 520 nucleotides. The variations at the 5′ end of the gene do not alter the structural ER protein because the translation initiation site is located within the shared sequence of exons 1 and 1′. Thus, both ER transcripts encode the same protein of 595 amino acids with a predicted molecular weight of 66 182 daltons. The presence or absence of ER protein in breast cancer traditionally has been determined by hormone-binding assay in cytosol extracts of tumor tissue homog-

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See "Notes" section following "References."

enates (*7*). The sensitivity of the hormone-binding assay is 3 fmol/mg cytosol protein, and tissues with values above 10 fmol/mg are generally considered ER-positive (*8*). In breast cancer patients, about two thirds of the tumors are ER-positive and one third are ER-negative (*9,10*). About 60% of ER-positive tumors, but only 5% of ER-negative cancers, respond to endocrine therapy. Stated in the opposite way, nearly all ER-negative and about 40% of ER-positive cancers are resistant to endocrine therapy (*11-14*).

The molecular mechanisms responsible for the ER-negative phenotype and the resistance to endocrine therapy are poorly understood. In search of the underlying mechanisms, several groups of investigators have examined the ER at the main levels of cellular expression, i.e., at the genomic level, as messenger RNA (mRNA) transcriptional product, and as protein. With regard to the genomic level, Southern blot analysis failed to show major rearrangements or deletions of the ER gene in ER-negative breast cancers (*15,16*). A more detailed analysis using polymerase chain reaction (PCR) amplification and restriction endonuclease digestion revealed a complete set of eight exons of normal sizes in ER-negative tumors indistinguishable from those seen in ER-positive tumors (*17*). These findings ruled out deletions/insertions of the ER gene greater than about 20 nucleotides in length but left open the possibility of point mutations in the coding region as the cause of the ER-negative phenotype. In this study, we examined the entire coding region of the ER gene in 118 ER-positive and 70 ER-negative primary breast cancers to determine whether small deletions/insertions or point mutations might account for the ER-negative phenotype.

Patients and Methods

Patients

The study is based on 188 women with primary invasive breast cancer who were treated at Vanderbilt University Medical Center, Nashville, Tenn., between 1982 and 1991. All patients had tumors of sufficient size (≥1.0 cm) to allow multipoint hormone-binding analyses of ER and progesterone receptor and extraction of DNA in addition to routine histopathologic studies. Demographic and clinical data were obtained from patients' records, with follow-up information provided by the Vanderbilt Tumor Registry, in accordance with the guidelines of the Institutional Review Board for the Protection of Human Subjects. Patients with a family history of breast cancer had one or more first-degree relatives and/or second-degree relatives with breast cancer. Disease stage was determined using the tumor–node–metastasis (TNM) classification (*18*).

Tissue Samples, Ligand Binding, and Immunohistochemical Assays of Steroid Receptors

All breast cancer biopsy specimens were examined in the Surgical Pathology Laboratory of Vanderbilt University Medical Center. After establishing the diagnosis of infiltrating breast cancer on frozen tissue sections, additional sections were obtained for immunohistochemical analysis of ER and progesterone receptor as previously described (*19*). A portion of tumor tissue was then fixed in formalin and embedded in paraffin for routine histopathologic examination. The remainder of the tumor tissue was stripped of adherent fat and frozen at –70 °C for biochemical studies.

The frozen tumor specimens were pulverized in liquid nitrogen. The resulting fine powder was suspended in low-salt buffer (50 m*M* Tris [pH 7.4], 1 m*M* EDTA, 10% glycerol, and 1 m*M* monothioglycerol) and centrifuged for 30 minutes at 100 000*g* and 4 °C. The supernatant cytosol was used for hormone-binding assays as described previously (*19*); the pellet was used to extract DNA by standard methods (*20*).

ER Gene Analysis

Oligonucleotide primers were prepared in the Biosynthesis Laboratory of the Department of Molecular Biology, Vanderbilt University, using an Applied Biosystems (Foster City, Calif.) DNA synthesizer. The primers were designed on the basis of published sequences to amplify exons 2 to 7 and the coding regions of exons 1 and 8 (*5,21,22*). Specifically, the following primer pairs were used for exon 1: 1A (5′-GTTTCTGAGCCTTCTGCCCTG), 1B (5′-TAGGGGAGGCCGGTCTG ACCGTAGA); 2A (5′-ATGGCCCTACTGCATCAGATCCAA), 2B (5-TTGGAGCCGAACGC CGCAGCCT); 3A (5′-TCTACGGTCAGACCGGCCT), 3B (5′-GCGCGG-GTACCTGTAGA ATG); exon 2: 4A (5′-CCC-AGGCCAAATTCAGATAA), 4B (5′-CGT-TTTCAACACACTATTA C); exon 3: 5A (5′-TGT-CCTCTTGCTTTTAATAG), 5B (5′-TGGGAG-AGATGTACCTACCA); exon 4: 6A (5′-TTCAC-CTGTGTTTTCAGGGA), 6B (5′-GCTGCGCTT-CGCATTCTTAC); exon 5: 7A (5′-CTTGCT-TGTTTTCAGGCTTTG), 7B (5-TACAGCCAG-GTCACTTACCT); exon 6: 8A (5′-TGCTATGTT-TTCATAGGAAC), 8B (5′-CTTGTGTTATCA-ACTCACCA); exon 7: 9A (5′-CTGCGCATTCAG-GAGTGTAC), 9B (5′-GAAGCCCAGAGATGC-CTCAC); and exon 8: 10A (5′-CTGTGT-CTTCCCACCTACAG), 10B (5′-CGTGTGGGA-GCCAGGGAGCT). A 36-base pair GC-clamp was attached to the 5′ end of each sense primer for denaturing gradient gel electrophoresis (*23*).

Genomic DNA (0.5 μg) was amplified through 36 cycles in a 100-μL volume containing 10 m*M* Tris–HCl [pH 8.3], 50 m*M* KCl, 4 m*M* $MgCl_2$, 200 μ*M* each of the four deoxyribonucleotides, *Taq* polymerase (2 U; Promega Biotec, Madison, Wis.), and each oligonucleotide at 25 μ*M*. Amplification conditions consisted of an initial denaturing step at 95 °C for 5 minutes, annealing at 62 °C for 1 minute, and polymerization at 72 °C for 1 minute, followed by 36 cycles at 95 °C for 30 seconds, 62 °C for 1 minute, and 72 °C for 6 minutes with a 30-second increment per 10 cycles in polymerization time. An annealing temperature of 67 °C was used for oligonucleotide pairs 7, 9, and 10 to reduce the number of nonspecific fragments. The polymorphic *Pvu*II restriction site in intron 1 was analyzed using PCR primers reported previously (*17*). A sample of each PCR mixture was size fractionated by electrophoresis in a 1.5% agarose gel and visualized by ethidium bromide staining.

Denaturing Gradient Gel Electrophoresis

Polyacrylamide electrophoresis of the amplified DNA (15-20 μL) was carried out using a denaturing gradient gel apparatus (Green Mountain Laboratory Supply, Waltham, Mass.). Gels contained 6.5% acrylamide in TAE buffer (40 m*M* Tris–acetate and 1 m*M* EDTA [pH 8.0]) with linearly increasing gradients of denaturants formamide and urea (*24*). The denaturant concentrations ranged from 30%-70% (100% denaturant corresponds to 40% formamide and 7 *M* urea). Gels were submerged in circulating TAE buffer at 60-65 °C, and DNA samples were electrophoresed at constant voltage (150 V) for 4-6 hours, depending on size and melting behavior of the amplified fragments. After electrophoresis, the gels were stained with ethidium bromide and photographed under UV light.

Single-Strand Conformation Polymorphism Analysis

The single-strand conformation polymorphism (SSCP) analysis was performed on PCR-amplified genomic DNA to screen for mutations in exon 1 of the ER gene (*25*). For each PCR reaction, both primers were 5′-end–labeled with [γ-^{33}P]adenosine triphosphate (ATP) (Amersham Corp., Arlington Heights, Ill.). Amplification conditions consisted of an initial denaturing step at 95 °C for 5 minutes, annealing at 62 °C for 1 minute, and polymerization at 72 °C for 1 minute, followed by 30 cycles at 95 °C for 30 seconds, 62 °C for 1 minute, and 72 °C for 6 minutes, with a 30-second increment per 10 cycles in polymerization time. Optimal electrophoretic separation was obtained on 5% polyacrylamide gels in TBE buffer (90 m*M* Tris–borate and 2 m*M* EDTA [pH 8.4]) containing 10% glycerol at 50 W for 4.5 hours at 4 °C.

Direct DNA Sequencing

DNA samples showing heteroduplexes on denaturing gradient gel electrophoresis or abnormal SSCP patterns were further analyzed to determine the exact location of each mutation. For this purpose, genomic DNA was amplified and each PCR product was purified through a GlassMax spin cartridge for cycle sequencing (GIBCO BRL, Grand Island, N.Y.) with primers labeled using [γ-^{33}P]ATP. The primers used for sequencing were the same as those used for PCR amplification. Addition-

al primers were synthesized to ensure complete sequencing of both strands of each PCR fragment. The sequencing reaction products were electrophoresed on a 6% polyacrylamide sequencing gel. After drying the gel, it was exposed to Kodak X-OMAT AR 5 film for 1-5 days.

Restriction endonucleases were selected to confirm the polymorphic sites in exons 1 and 8 and intron 1. The entire PCR product (100 μL) was ethanol precipitated and resuspended in 12 μL distilled H_2O for digestion. The samples were completely digested with *Hpa*II/*Msp*I (codon 10, exon 1), *Bst*UI (codon 87, exon 1), *Dsa*I (codon 594, exon 8), and *Pvu*II (intron 1) separated by electrophoresis in a 4% agarose gel (NuSieve GTG; FMC BioProducts, Rockland, Me.) and visualized by ethidium bromide staining.

Statistical Analysis

The data were assessed statistically by contingency-table analyses. The genotype was classified as homozygous 00, heterozygous 01, and homozygous 11 for each of the polymorphic alleles. The frequency distribution of patients in each subgroup was analyzed using Mantel's chi-squared test for trend (*26*). Confidence intervals for proportions were calculated using the method of Fleiss (*27*). Suppose that a gene has two alleles—one with frequency π and the other with frequency $1 - \pi$. Let n_{00}, n_{01}, and n_{11} denote the number of patients who are classified as homozygous 00, heterozygous 01, and homozygous 11, respectively, and let n denote the total number of patients. Then, under the null hypothesis of the Hardy–Weinberg equilibrium (*28*), the maximum likelihood estimate of π is $= (2n_{00} + n_{01})/2n$, and the maximum likelihood estimates of the probabilities that a patient will be homozygous 00, heterozygous 01, or homozygous 11 are π^2, $\pi 2(1 - \pi)$, and $(1 - \pi)^2$, respectively. We assessed the plausibility of the Hardy–Weinberg equilibrium by comparing these estimates with those associated with an unrestricted multinomial distribution, using a likelihood-ratio test (*29*).

Results

We examined the coding region of the ER gene in 118 ER-positive and 70 ER-negative primary breast cancers. We amplified exons 1 through 8 in all tumors and searched for deletions/insertions and mutations by agarose gel electrophoresis, denaturing gradient gel electrophoresis, and DNA sequencing. However, we were unable to examine exon 1 of the ER gene with the denaturing gradient gel electrophoresis technique. The reason is the unusually high GC content (70%) of exon 1, which interferes with adequate melting of the double-stranded DNA fragment in the denaturing gradient gel. Since GC-rich sequences, especially CpG dinucleotides, are hotspots for mutations in other genes, we used the SSCP method to investigate exon 1 for the presence of mutations.

Both ER-negative and ER-positive tumors contained neutral polymorphisms in codon 10 [TCT→TCC (Ser)], codon 87 [GCG→GCC (Ala)], codon 243 [CGC→CGT (Arg)], codon 325 [CCC→CCG (Pro)], and codon 594 [ACA→ACG (Thr)] in addition to a polymorphic site in intron 1 (Fig. 1). The polymorphic sites in codons 10, 325, and 594 have not been described previously. We examined the allele distribution in all haplotypes (Table 1). The distribution followed the Hardy–Weinberg rules for codons 10, 87, and 243 and intron 1. In contrast, the allele distribution for the polymorphic sites in codons 325 and 594 indicated a linkage disequilibrium (P = .003 and .017, respectively). There was no correlation of any of the polymorphic alleles with the ER phenotype or other parameters, including tumor type, size, grade, or stage. However, the polymorphism in codon 325 showed a strong association with a family history of breast cancer (P = .0005). This association was present both in premenopausal and postmenopausal patients (Table 2).

Despite extensive searching in exons 1 through 8, we found no deletions/insertions and only two missense mutations in the same ER-negative tumor. One mutation was in exon 1, codon 69 [AAC (Asn)→AAG (Lys)] and the second in exon 5, codon 396 [ATG (Met)→GTG (Val)] (Fig. 2). Analysis of normal peripheral lymphocyte DNA from the patient showed wild-type ER sequence, indicating that the tumor contained two somatic mutations. The patient had node-negative, stage II breast cancer and was alive and well 10 years postmastectomy. The fact that we observed only two mutations in 188 tumors (1%) indicates that missense mutations in the ER gene are rare in primary breast cancer.

Discussion

One third of all breast cancers are ER-negative, and mutations in the coding region of the ER gene have been implicated as a possible underlying cause of the ER-negative phenotype (*10-14,30,31*). However, our search of exons 1 through 8 of the ER gene in 188 breast cancers identified only two missense mutations in exons 1 and 5, both of which were found in the same ER-negative tumor. This means that point mutations in the ER gene coding region occur in 1% of primary breast cancers, a percentage far lower than that observed for the p53 gene (22%) with the same screening technique in the same group of tumors (*32*). Since the size and complexity of the ER gene is similar to that of the p53 gene, the question that arises is how to explain the difference in mutational frequency between the two genes. It has been shown that genes of similar size and complexity do not necessarily exhibit similar frequencies of mutational events (*33*). This apparent nonrandomness in the spectrum of genetic alterations indicates that the frequency of mutational lesions may be influenced by the local DNA sequence environment. Stated in more general terms, the nonrandomness of mutations suggests the existence of endogenous mechanisms of mutagenesis as distinct from the, until now, better characterized exogenous causes, such as radiation or chemical mutagens (*34*). Alternatively, the rate of repair of damaged DNA at individual nucleotides is highly variable and sequence dependent, suggesting that DNA repair efficiency may also contribute to the difference in mutational frequency between the ER and p53 genes (*35,36*).

We identified two somatic mutations in an ER-negative tumor. One mutation was in codon 69 in the N-terminal region of the ER gene that encodes an amino acid in one of the two transactivation domains, AF1, of the receptor (*37,38*). The other mutation was in codon 396, which is located in a region of the hormone-binding domain that is highly conserved among members of the steroid receptor family (*39,40*). The second mutation consisted of an A→G substitution, changing methionine to valine. Both human and chicken ER contain methionine at codon 396. However, the corresponding amino acid in *Xenopus* and rainbow trout ER is valine and isoleucine, respectively (*39,40*). Since wild-type *Xenopus* ER contains valine in this position, it is unlikely that a mutant valine in codon 396 of the human ER would alter the receptor function.

McGuire et al. (*41*) identified a missense mutation in codon 296 [CTC (Leu)→CCC (Pro)] of two breast cancers

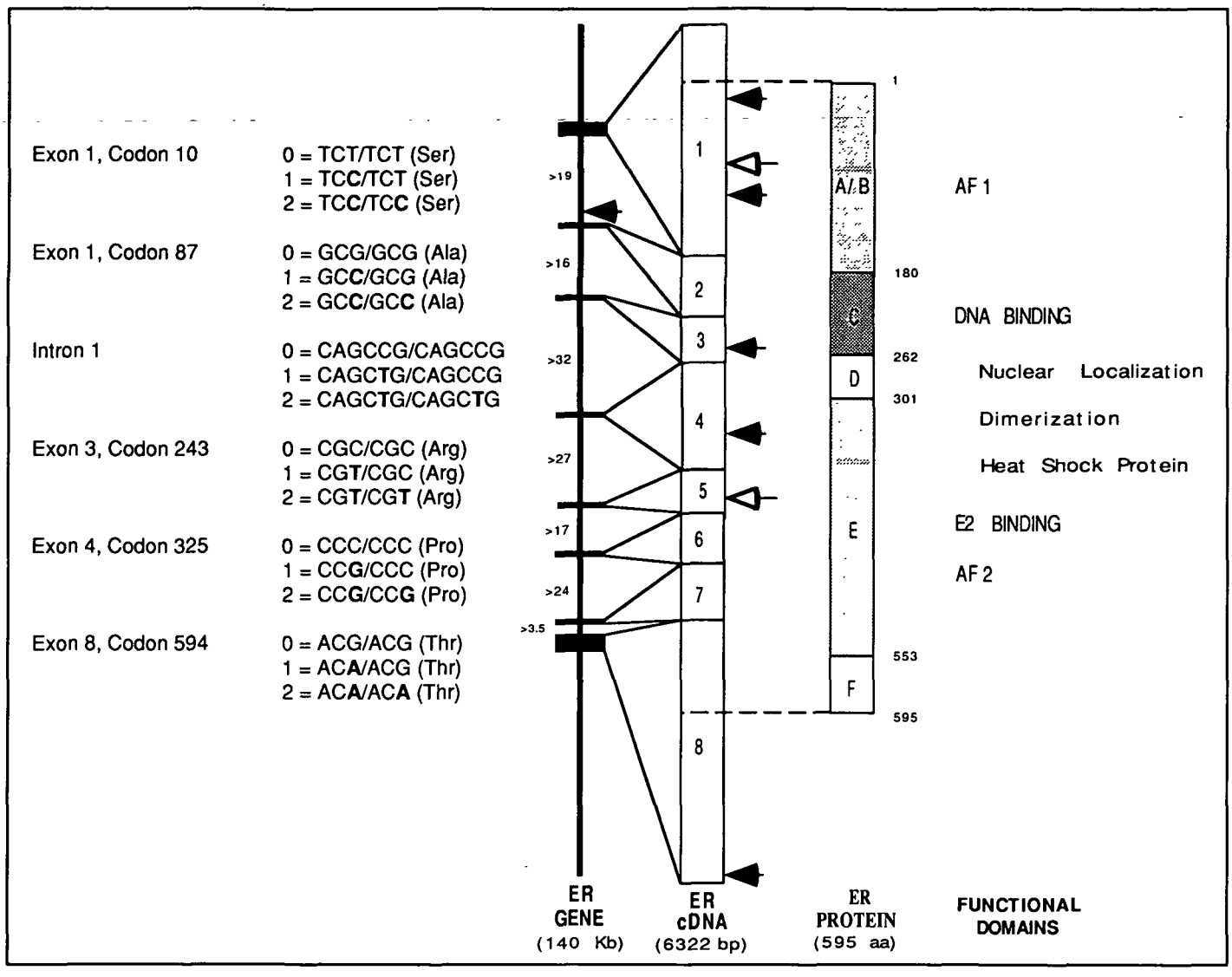

Fig. 1. Diagram of the ER gene, cDNA, and protein with functional domains, polymorphic sites, and mutations. The polymorphic sites are indicated by closed arrows, and the corresponding genotypes are listed on the left with the less common alleles indicated in bold characters. Note that the published wild-type sequence (*47*) of the ER cDNA lists the less common allele ACA for codon 594 instead of ACG found in the majority of individuals. Two missense mutations are indicated by open arrows in codons 69 and 396.

Table 1. Distribution and frequencies of ER gene polymorphisms in breast cancers (n = 188)

	Genotypes						Allele frequency (95% CI)		
	Observed			Expected†			Allele 0	Allele 1	
Polymorphism*	00	01	11	00	01	11			*P*‡
1	52	103	33	57.0	93.0	38.0	0.55 (0.50-0.60)	0.45 (0.40-0.50)	.14
2	165	22	1	164.8	22.5	0.8	0.94 (0.91-0.96)	0.06 (0.04-0.09)	.78
3	38	106	44	44.1	93.9	50.0	0.48 (0.43-0.54)	0.51 (0.46-0.57)	.08
4	181	7	0	181.1	6.9	0.1	0.98 (0.96-0.99)	0.02 (0.01-0.04)	.72
5	135	53	0	138.7	45.5	3.8	0.86 (0.82-0.89)	0.14 (0.11-0.18)	.003
6	129	47	12	123.7	57.6	6.7	0.81 (0.77-0.85)	0.19 (0.15-0.23)	.017

*1 = exon 1, codon 10; 2 = exon 1, codon 87; 3 = intron 1; 4 = exon 3, codon 243; 5 = exon 4, codon 325; 6 = exon 8, codon 594.
†Expected genotype frequency, assuming Hardy–Weinberg equilibrium.
‡Derived with respect to the null hypothesis of Hardy–Weinberg equilibrium.

and a missense mutation in codon 303 [AAG (Lys)→AGG (Arg)] of another tumor. Kamik et al. (*42*) examined the ER complementary DNA (cDNA) by SSCP in 20 tamoxifen-resistant and 20 tamoxifen-sensitive breast cancers. They observed a missense mutation in codon 352 [GAG (Glu)→GTG (Val)] of a tamoxifen-sensitive tumor. Since the tumor responded to the antiestrogen, the amino acid change did not appear to affect the receptor function. Two mutations

Table 2. Correlation of ER gene polymorphism in codon 325 with familial breast cancer (n = 188)

	Family history, No. of patients (%)			No family history, No. of patients (%)			
Age, y	Allele 0	Allele 1	Total	Allele 0	Allele 1	Total	*P*
<50	3 (33.3)	6 (66.7)	9	43 (84.3)	8 (15.7)	51	.001
≥50	12 (48.0)	13 (52.0)	25	76 (73.8)	27 (26.2)	103	.013
All ages	15 (44.1)	19 (55.9)	34	119 (77.3)	35 (22.7)	154	.0005

were detected in the group of tamoxifen-resistant tumors. The first was a single base-pair deletion in codon 432 [TCA→CA], which was found in a tamoxifen-resistant metastatic tumor but not in the primary tumor from the same patient. The second mutation involved a substitution of 47 nucleotides (1271-1318) of exon 6 by 42 nucleotides (1148-1190) of exon 5 in a metastatic tumor. The remaining 18 tamoxifen-resistant tumors did not contain mutations in any of the eight exons of the ER cDNA. Overall, these results indicate that mutations in the coding region of the ER gene occur at a low frequency in primary breast cancers. Thus, they do not account for the ER phenotype of the majority of ER-negative or tamoxifen-resistant ER-positive breast cancers. However, the finding of ER gene mutations in two metastatic tumors in the study of Karnik et al. (*42*) leaves open the possibility that ER gene alterations may be more frequent in metastatic breast cancers.

Similar results were obtained in studies of the androgen receptor gene in prostate cancer. In one study (*43*), analysis of the androgen receptor gene in 27 prostate cancers did not show any evidence of receptor mutations. In another study, Newmark et al. (*44*) found only one mutation in 26 prostate cancers. It can be concluded that, although the ER and the androgen receptor are key molecules in breast and prostate development, mutations of the respective receptor genes do not substantially contribute to aberrant cell growth in breast or prostate cancer.

If the ER-negative phenotype of breast cancers cannot be accounted for by mutations in the coding region of the ER gene, other causes need to be identified. One such cause may be the presence of variant species of ER mRNA containing precise deletions of individual exons that occur in ER-negative breast tumors and breast cancer cell lines (*45,46*). Another mechanism that could alter the level of ER gene transcription is methylation of GC-rich CpG islands. A study of breast cancer cell lines showed extensive methylation of the *Not*I site in the 5′ CpG island of the ER gene in ER-negative cells but not in ER-positive cells. In addition, the ER-negative cells had higher levels of DNA methyltransferase than the ER-positive cells, suggesting an increased capacity to methylate DNA, which might account for the low level of ER mRNA in the former cells (*47*). Whether these observations on methylation-dependent ER expression can be extended from cell lines to tumors remains to be determined. Finally, the existence of mutations in transcriptional regulatory elements (either upstream from the coding sequences or contained within introns) cannot be ruled out.

We identified six polymorphic sites in the ER gene and determined the allele frequencies for each haplotype (Table 1). There was no correlation of any of the polymorphic alleles with the ER phenotype or other clinicopathologic parameters, including tumor type, size, grade, or stage. However, the polymorphism in codon 325 showed a strong association with a family history of breast cancer (*P* = .0005; Table 2). In addition, the allele distribution for codon 325 indicated a linkage disequilibrium. It is intriguing that a possible linkage of the ER gene locus with familial breast cancer was also observed by Zuppan et al. (*48*) in genetic linkage studies of inherited breast cancer. For one extended family with eight patients who had late-onset disease, one ER haplotype was consistently coinherited with breast cancer, yielding a +1.85 LOD score for linkage at zero recombination (*48*). The haplotype was defined by restriction markers using *Xba*I, *Sac*I, and *Hin*dIII. Codon 325 is not part of a recognition sequence for *Xba*I, *Sac*I, or *Hin*dIII. Recently, linkage of the ER gene locus to breast cancer was observed

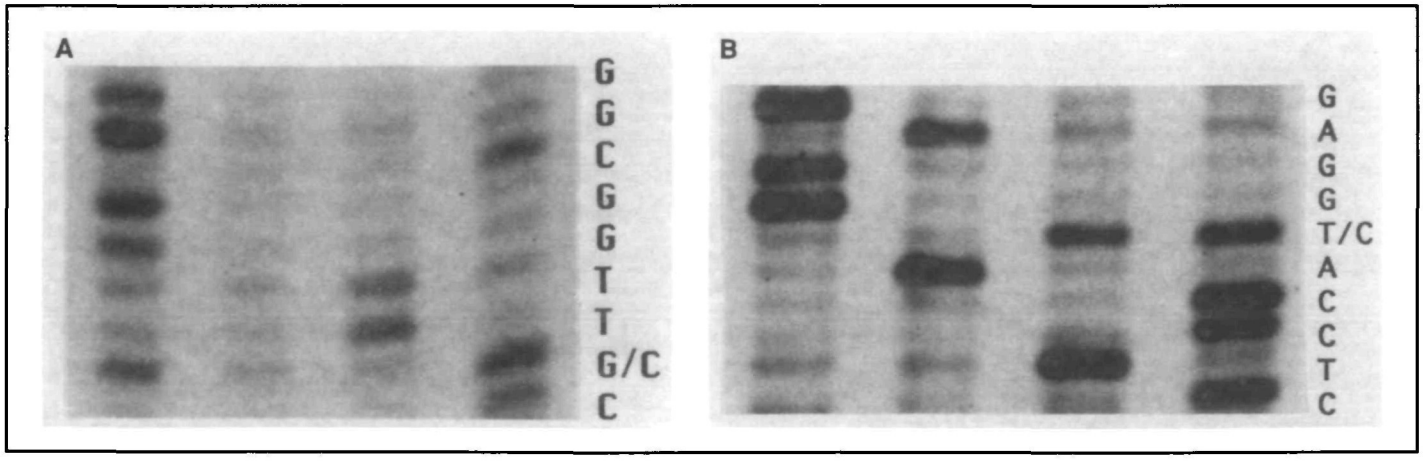

Fig. 2. DNA sequence of missense mutations. A) DNA sequencing of mutant ER gene shows a G→C mutation in the antisense strand [AAC (Asn)→AAG (Lys)] in codon 69. The simultaneous presence of G and C indicates that the tumor is heterozygous for the mutation. The mutation leads to obliteration of a *Bst*UI restriction site, which was used as an independent confirmation of the genetic alteration. B) DNA sequencing of mutant ER gene showing a T→C mutation in the antisense strand [ATG (Met)→GTG (Val)] in codon 396. This mutation leads to obliteration of an *Nco*I restriction site.

in another family—involving four premenopausal and three postmenopausal patients (King MC: personal communication). In light of the correlation reported by King's group, as well as in our findings in 188 breast cancer patients, it will be interesting to further investigate the possible linkage of the ER gene locus to hereditary breast cancer.

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Notes

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